Aspects of the ecology and conservation of the Growling Grass Frog* *Litoria raniformis* in an urban-fringe environment, southern Victoria

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ABSTRACT

Populations of the threatened Growling Grass Frog *Litoria raniformis* in metropolitan Melbourne currently occur in human-made habitats and there is little information on their current status. We conducted systematic field surveys at 28 waterbodies within a population distributed over agricultural land in the Pakenham area, on the south-eastern urban-fringe of Melbourne, Victoria, during the 2005/06 breeding season. Our aim was to identify key areas for the conservation of *L. raniformis*. We recorded *L. raniformis* at 14 waterbodies where we marked 31 males and 16 females. Occupied waterbodies had a greater proportion of submerged vegetation, a higher pH, and were situated close to other occupied waterbodies, generally < 200 m. The adult male and female population size was estimated at 45 ± 10 (± S.E.) and 23 ± 8 individuals, respectively. There was high site fidelity at occupied waterbodies (> 80% recapture rates). Recruitment was recorded in only two waterbodies. Two males and four females were recorded moving 15 - 20 m between adjacent waterbodies, whereas one additional male moved 130 m. Dispersal and movement corridors important for the persistence of *L. raniformis* in the study area include agricultural drains and Gum Scrub Creek. High priority conservation areas are waterbodies situated in close proximity to the creek and Melbourne – Bairnsdale railway reserve, and clusters of occupied waterbodies to the south.

Key words: endangered species, mark-recapture study, habitat model, population estimation

Introduction

The Growling Grass Frog *Litoria raniformis* was once widely distributed across south-eastern Australia, including Tasmania (Littlejohn 1963, 1982; Hero *et al.* 1991), but has declined markedly across much of its former range. Historically, the species was recorded from most regions of Victoria, with the exception of the western mallee and eastern alpine areas (Littlejohn 1963, 1982; Hero *et al.* 1991). The range of *L. raniformis* has contracted dramatically over the past two decades and in many areas, particularly in south and central Victoria, populations have experienced serious declines and local extinctions (Pyke 2002). On the basis of the range contraction and local declines, the species is currently listed as 'Vulnerable' under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and 'Endangered' under the *Advisory List of Threatened Vertebrate Fauna in Victoria* (DSE 2003). It is also listed as threatened under the Victorian *Flora and Fauna Guarantee Act 1988* (FFG Act). A draft 'Action Statement' under the FFG Act has been developed for *L. raniformis*, which outlines the current threats to the species, and provides specific actions to protect and, where possible, increase populations in the future (Robertson 2003).

Within extant populations, whether situated in natural or urban landscapes, *L. raniformis* inhabits permanent or semi-permanent, still or slow flowing waterbodies, often with emergent aquatic vegetation (e.g. lakes or reservoirs, swamps, slow-flowing sections of streams, lagoons, farm dams and old quarry sites) (Hero *et al.* 1991; Barker *et al.* 1995; Ashworth 1998; Cogger 2000). A detailed review of current information on the biology of *L. raniformis* is provided in Pyke (2002).

Populations of *L. raniformis* currently occur on the urban-fringe of metropolitan Melbourne, Victoria, and recent surveys have revealed several relatively large populations, including widely distributed populations across agricultural land in the Pakenham area, located on the south-eastern urban-fringe of Melbourne (Hamer and Organ 2006a). Additional extant populations that occur within metropolitan Melbourne inhabit a range of natural and artificial waterbodies at the Werribee Sewage Treatment Plant, throughout the Merri Creek catchment north of Melbourne, and in several locations in the western suburbs (Robertson *et al.* 2002; Heard *et al.* 2004; Organ 2005a, Hamer and Organ 2006b). Like the closely-related Green and Golden Bell Frog *L. aurea*, *L. raniformis* has the ability to colonise human-made habitats in the vicinity of large

*Referred to as the Southern Bell Frog in NSW

urban centres (White 1995), and currently, most of the core breeding sites around Melbourne occur in such environments. However, despite the close proximity of these populations to densely populated urban areas, there is little information on their ecology and conservation requirements. Many of these urban-fringe populations are in imminent threat of extinction from habitat loss, fragmentation and degradation due to urban development.

The objective of this study was to investigate aspects of the ecology of *L. raniformis* within a population that inhabits largely artificial habitats (i.e. farm dams) on agricultural land across the Pakenham Urban Growth Corridor on the south-eastern urban-fringe of Melbourne, Victoria. The objective had two components: (i) to document wetland occupancy patterns and habitat correlates; and (ii) to obtain key demographic information (e.g. population size and structure, dispersal) from a mark-recapture study conducted on the population. The wider aim was to identify key areas for the conservation of *L. raniformis* and to ensure future land use planning within the growth corridor is based on strategic ecological advice.

Methods

Study area

The study area was approximately 600 ha in size and located within a portion of the Pakenham Urban Growth Corridor, situated approximately 65 km south-east of the Melbourne Central Business District (Figure 1). This study area was specifically chosen because it was previously known to contain an extant population of *L. raniformis* (Timewell 2003; Organ 2004) and is currently undergoing a high level of urbanisation. It is therefore imperative that ecological information on the population is collected in order to guide conservation actions for the species within the growth corridor.

The study area was highly modified, supporting few areas of remnant native vegetation, and was primarily used for agriculture (i.e. cattle grazing). Gum Scrub Creek flows in a southerly direction across the study area, while several shallow drainage lines were also present (e.g. between Cardinia Road and Gum Scrub Creek, parallel to the Melbourne – Bairnsdale railway, and along roadsides). The proposed Pakenham Bypass (freeway) dissects the study area in an east-west fashion. The study area has been targeted for future urban growth, including residential development and associated infrastructure, and the human population is expected to double by 2030 (I.D. Consulting 2003).

Remnant indigenous vegetation in the study area has been mapped as swampy riparian complex and plains woodland/grassland mosaic (Oates and Taranto 2001). Swampy riparian complex occurs on wet flats and in drainage basins, which have mostly been drained and cleared for agriculture, and would once (pre-1750) have been widespread over the flatter areas of the study area. Woodland and indigenous grassland is largely absent, but would have occurred in areas of higher elevation.

Figure 1. Location of the study area, Cardinia Shire, southern Victoria.

Twenty-eight waterbodies within the growth corridor, all situated on privately-owned land, were selected for this study. Waterbodies were selected from an aerial photograph of the study area (scale 1:13130), primarily waterbodies where *L. raniformis* had been previously recorded during surveys undertaken to assess the proposed Pakenham Bypass, but also where it had not (Timewell 2003; Organ 2004). We aimed to include as many free-standing waterbodies as possible, and to include a wide range of waterbody types (i.e. varying size, shape, habitat structure, grazing pressure and spatial orientation in the landscape). We did not survey creeklines. We obtained permission from landholders for entry prior to conducting surveys on their property.

The sites were delineated into three spatial clusters: cluster A contained 10 waterbodies (A1 – A10); cluster B contained eight waterbodies (B1 – B8); and cluster C contained 10 waterbodies (C1 – C10) (Figure 2). Cluster A included waterbodies situated in close proximity (approximately 100 m) to the railway reserve in the northern portion of the study area. Cluster B included waterbodies situated in the vicinity of the Pakenham Bypass, whereas cluster C included waterbodies dispersed around Gum Scrub Creek, Lecky Road and Cardinia Road in the southern portion of the study area.

Habitat surveys

To determine the habitat use of *L. raniformis* in the study area, 26 waterbodies were surveyed for a suite of habitat variables (Table 1). Habitat variables were collected from the waterbodies on 20 - 22, 28 and 29 December 2005, 3 - 6, 16 and 31 January 2006, and 1 February 2006. Variables were recorded in 1 m^2 quadrats at seven equidistant points around each waterbody on the bank, 1 m from the water's edge, to sample the terrestrial vegetation surrounding waterbodies, and 1 m from the shoreline in the water to sample aquatic vegetation, water depth and water chemistry (Hamer *et al.* 2002a).

Vegetation/physiognomy

Area was measured in the field by pacing the length and breadth of each waterbody. The percentage cover of the vegetative structure (shrub, macrophyte, herb, grass, bare ground) was scored by eye within quadrats placed on the ground. The percentage cover of canopy, emergent, submerged and floating vegetation, algae and open water was also estimated by eye within quadrats placed in the water. Vegetation height was measured at the centre of each 1 m^2 quadrat, whereas water depth was measured at the centre of the quadrats placed in the water. Plant species were identified from Auld and Medd (1987) and Sainty and Jacobs (1994), or sent to the New South Wales National Herbarium for identification.

Water chemistry

Turbidity was measured using a turbidity tube (Waterwatch, East Melbourne, Victoria), whereas the remaining water chemistry parameters were measured using a Tracer Pocketester (LaMotte, Chestertown, MD, USA). The

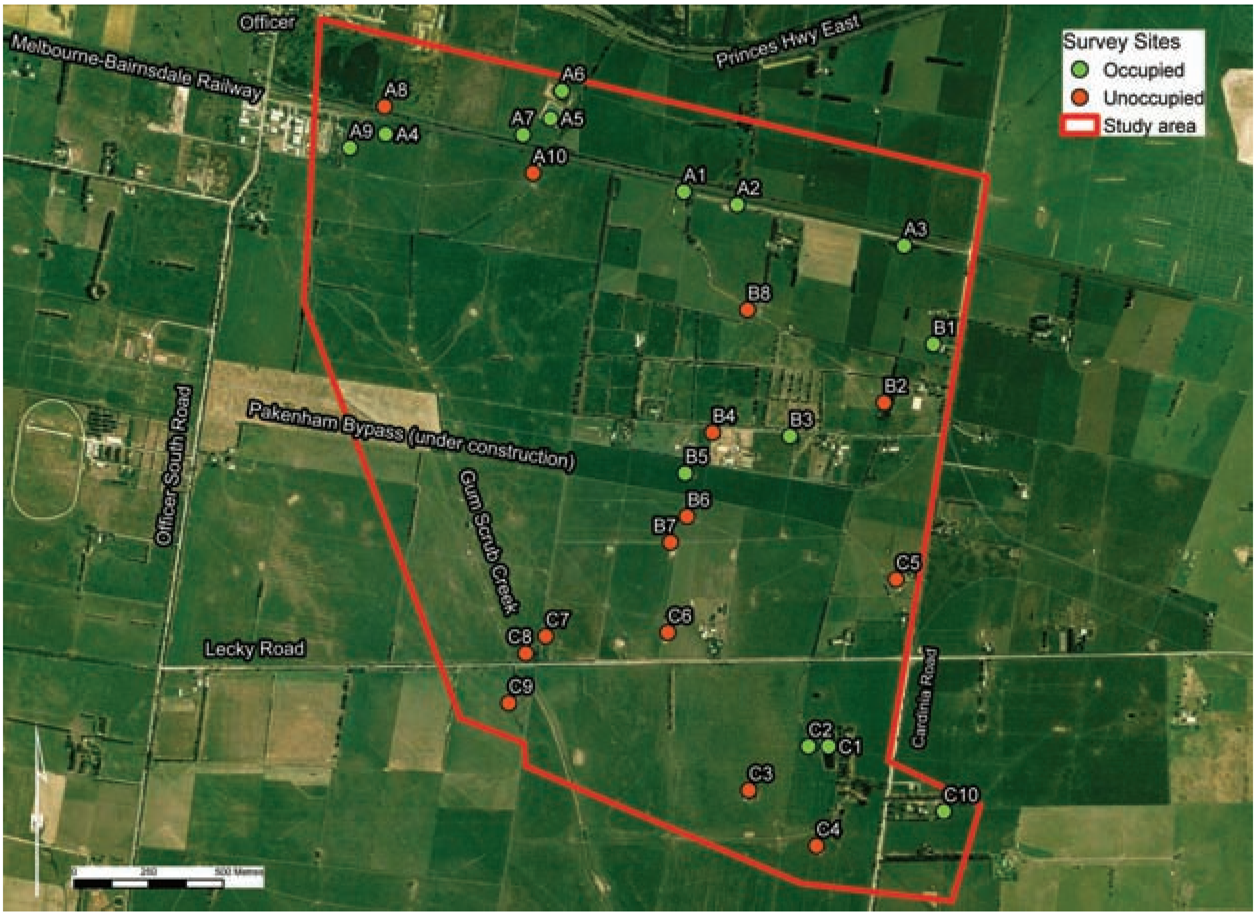

Figure 2. Location and occupancy status of the 28 survey sites.

Table 1. Description of the habitat variables recorded at 26 waterbodies.

Code	Description
DISTOCC	Distance to nearest occupied waterbody (m)
AREA	Waterbody area (m^2)
%CANOPY	% of waterbody covered by tree canopy
%EMERG	% of waterbody covered by emergent vegetation
%SUBMERG	% of waterbody covered by submerged vegetation
%FLOAT	% of waterbody covered by floating vegetation
%ALGA	% of waterbody surface or bottom covered by algae
%OPEN	% of waterbody with open water
BANKVEGHT	Vegetation height along the bank (cm)
SHOREVEGHT	Vegetation height 1 m from the shoreline (cm)
SHOREDEPTH	Water depth 1 m from the shoreline (cm)
SHORESAL	Salinity (ppm) at 1 m from the shoreline
SHOREPH	pH at 1 m from the shoreline
SHORECOND	Conductivity (mS) at 1 m from the shoreline
SHORETDS	Total Dissolved Solids (ppm) at 1 m from the shoreline
TURBIDITY	Turbidity (ntu) at 1 m from the shoreline
%BANKGRASS	% of bank covered by grass
%BANKHERB	% of bank covered by herbaceous vegetation
%BANKSHRUB	% of bank covered by shrubs
%BANKMACRO	% of bank covered by macrophytes
%BANKBARE	% of bank that is bare
GRAZING	Grazing pressure (high, moderate, low, none)
FISH	Observations of plague minnow

readings were obtained from a 150 ml sample collected within 10 cm of the water surface at the centre of the quadrat. Seven readings were taken at each site to obtain an average for the waterbody.

Grazing pressure

We scored grazing pressure because the land surrounding most waterbodies was being grazed by cattle and this may affect the use of waterbodies by *L. raniformis*. We used the categories of Robertson *et al.* (2002): High = significant trampling such that the bank was de-vegetated; Moderate = trampling frequent but bank-side vegetation remained in place; Low = trampling evident but it did not disturb the bank-side vegetation; None = no trampling evident.

Plague minnow

Observations of the exotic plague minnow *Gambusia holbrooki* in waterbodies were primarily determined through visual inspection. This predatory species is known to eat the eggs and tadpoles of *L. aurea* (Morgan and Buttemer 1996; Pyke and White 2000). It is easily observed in waterbodies as the fish school in large numbers close to the water surface and near shallow edges. The use of fish traps facilitated observations of fish at six waterbodies (see Field Surveys).

Spatial pattern

The spatial pattern of waterbody occupancy in the study area was determined by measuring the distance (m) to the nearest occupied and nearest unoccupied waterbody, from their respective edges, using a scaled aerial photograph.

Field surveys

Systematic surveys for *L. raniformis* were conducted at the 28 waterbodies between November 2005 and February 2006 during the core breeding season of this species (Barker *et al.* 1995); survey period 1: 16 – 30 November 2005; survey period 2: 19 December 2005 – 6 January 2006; and survey period 3: 23 January – 9 February 2006. Each waterbody was surveyed nine times, comprising three individual surveys within the three survey periods. Survey periods were arranged two weeks apart and the three site clusters were surveyed repeatedly over three consecutive weeks. Waterbodies within each cluster were surveyed over one separate night so that the entire survey area was sampled over three nights. Sites A9, A10, C2 and C10 were surveyed eight, five, three and three times, respectively, because they were identified as potential habitat for *L. raniformis* following the initial site selection and/or the species was recorded opportunistically, and the sites were then added to the standard field survey program. Although we did not assess detection probabilities for adult *L. raniformis* within the study area, we consider our survey effort to be sufficient to detect the species with a high level of confidence because detection probabilities for *L. raniformis* in an adjacent region of Melbourne were estimated to exceed 0.99 and 0.90 when nine and three spotlight surveys were conducted, respectively (Heard *et al.* 2006).

The order of waterbodies surveyed within each cluster was chosen randomly to minimise bias, because time of night may influence frog activity. Nocturnal surveys comprised quiet listening at each waterbody for approximately 5 min. The advertisement call of male *L. raniformis* was imitated for several minutes to elicit a response from any adult males, followed by quiet listening for several minutes. A hand-held spotlight and head-mounted light was used to search for frogs on the banks, on floating vegetation and in areas of emergent vegetation. The surrounding terrestrial habitat within 10 m of the waterbody was also searched. The time spent surveying each site varied depending on size and habitat complexity, but was between 10 – 40 min.

Surveys for the tadpoles of *L. raniformis* were conducted at waterbodies where calling males were recorded: sites A1, A2, B4, B6 and C1, using 2 - 3 fish traps (45 cm long x 25 cm high x 25 cm wide, 15 cm long funnel at each end) placed in each waterbody. The traps have inverted ends that "funnel" aquatic organisms into the trap and are similar to funnel-traps used in other studies of bell frogs (Hamer *et al.* 2002a; Heard *et al.* 2006). Fluorescent glow-sticks were placed into each trap to attract tadpoles, although their effectiveness has not been tested. The traps were deployed on the 29 December 2005 and inspected and retrieved the following morning (within 12 h). Tadpoles were identified from Anstis (2002) and their developmental stage determined from reference to Gosner (1960). Fish species caught in the traps were also noted. Although we did not assess detection probabilities for *L. raniformis* tadpoles in the study area, Heard *et al.* (2006) estimated a probability of 0.35 of detecting tadpoles after a single survey. Therefore, our results may under-estimate the actual use of the five sites as breeding habitat. Active searching for metamorphs was also conducted during nocturnal surveys at sites where calling and/or tadpoles were recorded.

Mark-recapture study

Frogs were caught by hand during the nocturnal surveys and each placed into an individual plastic bag. Male frogs were identified by the presence of nuptial pads, whereas absence of this character denoted females (Pyke 2002). However, the absence of nuptial pads does not necessarily imply an individual is female, but may be a non-mature male. Frogs > 52.1 mm without nuptial pads were classed as female because the smallest male with nuptial pads we observed in the field was 52.1 mm. Snout-vent length (SVL) was recorded with dial calipers to the nearest 0.1 mm. Frogs were weighed to the nearest 0.25 g using a spring balance (Pesola).

To uniquely identify individuals, a Passive Integrated Transponder (PIT) microchip (Trovan Ltd., UK) was inserted into unmarked individuals if their snout-vent length (SVL) exceeded 40 mm, following the methodology of Christy (1996). All captured individuals were scanned using an electronic tag reader (Trovan). Juvenile frogs (< 40 mm SVL) were not marked. Frogs were released at the point of capture on the same night.

Data analysis

Habitat modelling

Logistic regression was used to model the presence or absence of *L. raniformis* at a waterbody (i.e. dependent variable) as a function of the habitat variables presented in Table 1 (i.e. independent variables). However, prior to model construction, the number of independent variables had to be reduced because of the high degree of co-linearity. For example, ponds with a high percentage of emergent vegetation had less open water and vice versa so it was inappropriate to include both measurements in the regression model. Consequently, a Principal Components Analysis (PCA) was used to reduce the number of continuous variables to a set of factors that were then rotated using the Varimax procedure to produce orthogonal (non-correlated) factors (Hair *et al.* 1998). Categorical variables (e.g. FISH, GRAZING) are not suited to PCA's and were not included. Components were extracted according to the eigenvalues (greater-than-one rule), which represent the amount of variance explained by the factor. The factor matrices were examined and those variables with the highest loadings on each component (> 0.6) were subsequently used to describe a particular factor dimension in the regression model. The rotated factor scores and two categorical variables were then included as independent variables in a logistic regression. We tested the null hypothesis that the β coefficient of each variable in the analysis did not differ significantly from zero.

Prior to analysis percent data were arcsine transformed and log(x) transformations were also used when appropriate. Data have been back-transformed for presentation purposes where means are given with ± 1 standard deviation (S.D.). The PCA and regression analysis was performed using *Statistica* software (StatSoft Inc., Tulsa, Oklahoma, USA).

Spatial pattern

Recent field studies have highlighted the importance of spatial pattern in the occupancy of waterbodies by bell frogs (Hamer *et al.* 2002a; Robertson *et al.* 2002; Heard *et al.* 2004). We therefore conducted an additional analysis using the spatial variable DISTOCC. A frequency histogram of the distance to the nearest occupied waterbody for occupied and unoccupied waterbodies was constructed to determine whether the waterbodies that *L. raniformis* occupied were spatially aggregated. A Student's *t*-test was used to compare the distances to the nearest occupied waterbody for all occupied and unoccupied waterbodies in the study area (n = 28). The distance to the nearest occupied waterbody was logarithmically transformed to meet the assumptions of normality.

Mark-recapture

The Bailey's Triple Catch method was used to estimate the size (N) ± 1 standard error (S.E.) of the *L. raniformis* population in the study area because the survey structure comprised three sampling periods. This estimator requires three samples and makes few assumptions (Begon 1979). Population size on the second sampling occasion (i.e. survey period 2) was estimated separately for male and

female captures. Estimates were obtained for survey period 2 because the highest number of recaptures was recorded at this time. We used mark-recapture data from the three survey periods in estimating the population size (*N*) as follows:

$N_2 = M_{21}(n_2+1)/(m_{21}+1)$

where M_{21} is the number of frogs marked in survey period 1 that "survive" to survey period 2 of r_1 frogs caught and marked in survey period 1:

$M_{21} = m_{31}(r_2+1)/(m_{32}+1) + m_{21}$

n_2 = number of frogs caught in survey period 2

m_{21} = number of frogs caught in survey period 2 that were marked in survey period 1

m_{31} = number of frogs caught in survey period 3 that were marked in survey period 1 but not captured in survey period 2

m_{32} = number of frogs caught in survey period 3 that were marked in survey period 2

r_2 = number of frogs caught in survey period 2

r_3 = number of frogs caught in survey period 3

Recapture rates were calculated for males and females across the three survey periods (i.e. total number of individuals recaptured in survey periods 1 – 3 divided by the total number of individuals marked in survey periods 1 – 3). We produced size frequency histograms separately for males and females over the three survey periods. Male and female SVL and mass were compared using Student's *t*-tests following logarithmic transformation of mass. Mean male and female SVL were compared among the three survey periods using parametric one-way Analysis of Variance (ANOVA). The Holm-Sidak method was used to determine where significant differences among the survey periods occurred.

All statistics are presented as means ± 1 standard deviation (S.D.), unless otherwise denoted.

Results

Occupancy

Spatial pattern

Litoria raniformis was recorded at 14 waterbodies in the study area, with an occupancy rate of 50% (Figure 2). The distance to the nearest occupied waterbody was significantly greater for unoccupied waterbodies (366 ± 273 m) than occupied ones (172 ± 150 m) (t = -2.61, P = 0.015, d.f. = 26). Sixty-four percent of occupied waterbodies were within 200 m of another occupied waterbody, whereas 71% of unoccupied waterbodies were greater than 200 m from the nearest occupied waterbody (Figure 3). Twenty-nine percent of occupied waterbodies were within 50 m of another occupied waterbody, whereas none of the

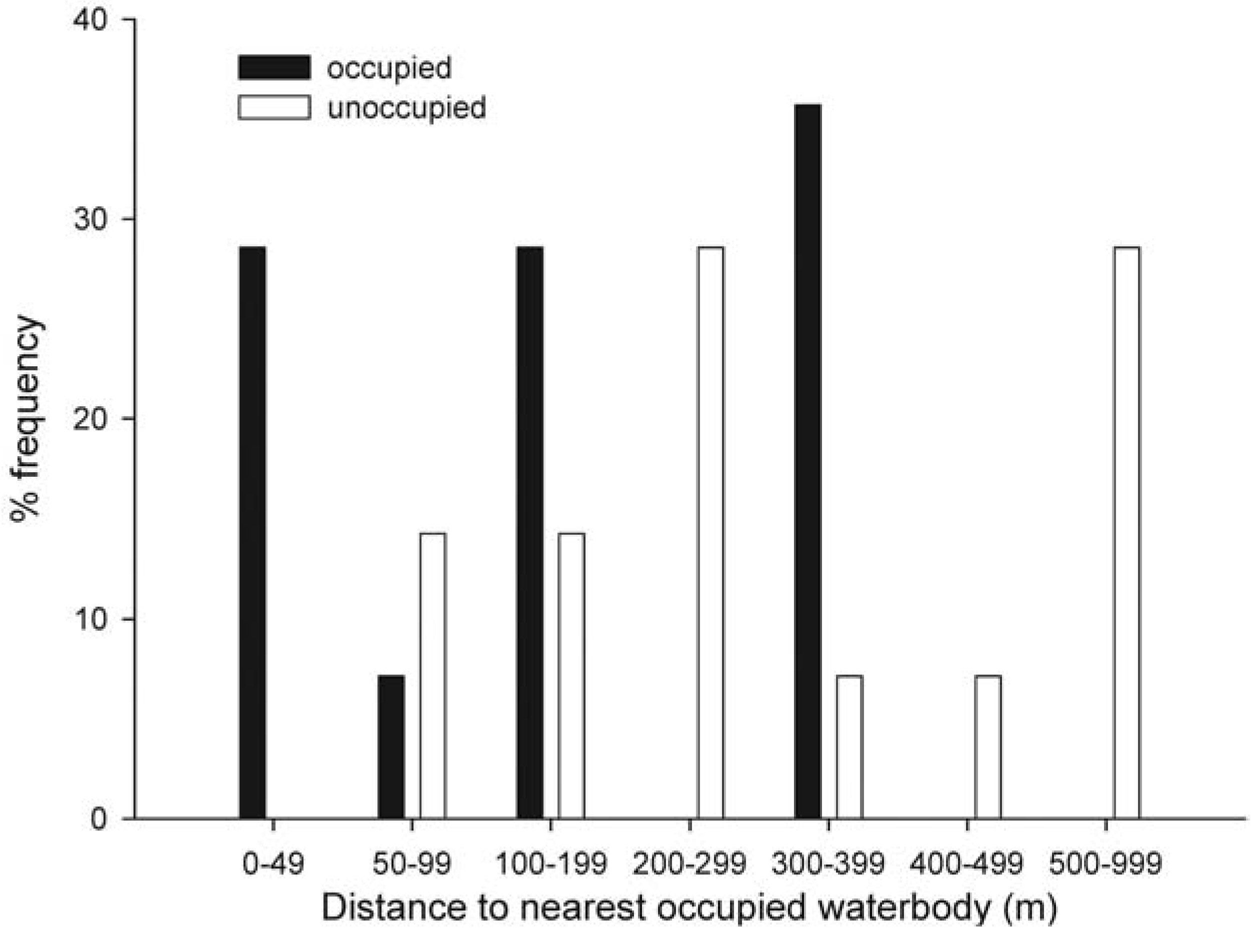

Figure 3. Frequency distribution of distance to the nearest occupied waterbody for occupied (n = 14) and unoccupied waterbodies (n = 14).

unoccupied waterbodies were within 50 m of an occupied waterbody. Therefore, there was significant clustering in the distribution of *L. raniformis* in the study area and unoccupied waterbodies were significantly isolated.

Habitat correlates

The first four factors from the PCA matrix accounted for 65.8% of the original variation. Factor 1 described waterbodies with a high percentage cover of open water and a correspondingly low cover of emergent and floating vegetation, and a low bank and shore vegetation height (26.1% variance explained). Factor 2 described waterbodies with low salinity, Total Dissolved Solids (TDS) and conductivity but which had a high percentage cover of grass on the banks (18.6% variance explained). Factor 3 defined waterbodies with a high percentage cover of submerged vegetation, high pH and that were close to waterbodies occupied by *L. raniformis* (11.7% variance explained). Factor 4 described waterbodies with a low percentage cover of herbaceous and macrophytic vegetation on the banks (9.4% variance explained). Mean values for each of the 21 continuous habitat variables recorded at the occupied and unoccupied waterbodies are presented in Table 2.

Logistic regression model

A model was created that could successfully predict the presence of *L. raniformis* at a waterbody with 85.7% accuracy. The logistic regression model was statistically significant (χ^2 = 17.81, P = 0.007, d.f. = 6) and there was no evidence for lack of fit (Hosmer – Lemeshow test: P = 0.862). The null hypothesis was rejected for factor 3 (Wald statistic = 6.5, P = 0.02). Examination of the mean values for the variables included on factor 3 in occupied and unoccupied sites showed that *L. raniformis* occupied waterbodies close to other occupied waterbodies, and waterbodies with a greater percentage cover of submerged vegetation and higher pH (Table 2). Waterbody occupancy was unrelated to observations of the plague minnow (Wald = 2.2, P = 0.15) or to grazing pressure (Wald = 0.4, P = 0.55).

Description of vegetation at occupied waterbodies

The dominant species of submerged vegetation at occupied waterbodies were the pondweeds *Potamogeton ochreatus*, *P. tricarinatus* and *P. pectinatus*, and the freshwater algae *Chara* spp., while the dominant floating vegetation included *Azolla pinnata* and *Ottelia ovalifolia*. Dense growth of *Potamogeton* spp. at many occupied waterbodies resulted in the appearance of 'rafts' of floating vegetation. Calling males were often observed on submerged and floating vegetation early in the study, although their use of this habitat and breeding activity appeared to decline when the vegetation died back from December onwards. Emergent and fringing vegetation, including bank macrophytes, was dominated by *Eleocharis sphacelata*, *E. acuta*, *Juncus* spp. and *Typha* spp. Grasses on the waterbody banks were dominated by exotic species such as *Agrostis capillaris*,

Table 2. Descriptive statistics for the 21 continuous habitat variables recorded at 26 waterbodies. See Table 1 for a description of the variables.

	Occupied (n = 14)				Unoccupied (n = 12)			
	Mean	S.D.	Minimum	Maximum	Mean	S.D.	Minimum	Maximum
DISTOCC*	171.7	149.9	15.0	394.0	365.7	273.1	65.0	945.0
AREA*	797.1	991.4	50.0	2800.0	342.8	291.1	50.0	1275.0
%CANOPY	2.0	5.9	0	21.4	0	0	0	0
%EMERGENT	19.7	21.6	0	57.9	17.4	28.6	0	70.0
%SUBMERG	31.9	31.6	0	95.0	5.9	14.8	0	51.0
%FLOAT	7.5	9.6	0	27.9	3.4	8.0	0	25.7
%ALGA	2.4	6.5	0	22.6	1.5	3.9	0	13.6
%OPEN	62.7	32.0	2.1	100	79.4	35.9	3.9	100
BANKVEGHT	29.6	27.6	1.6	90.3	28.3	24.4	0	66.5
SHOREVEGHT	12.4	17.9	0	59.1	18.5	32.8	0	78.9
SHOREDEPTH	32.0	16.8	0.8	64.3	21.4	9.3	8.9	44.7
SHORESAL	493.0	466.8	127.3	1708.0	315.1	203.9	135.7	763.8
SHOREPH	8.7	0.7	7.6	10.0	7.8	0.7	6.7	9.7
SHORECOND	999.5	949.6	259.6	3477.0	637.9	411.1	276.9	1537.4
SHORETDS	696.6	661.2	181.6	2420.0	443.5	287.2	191.5	1072.3
TURBIDITY	34.2	53.3	0	172.8	195.7	195.3	10.3	500.0
%BANKGRASS	63.3	25.2	10.3	97.1	51.5	25.4	1.9	90.0
%BANKHERB	11.1	6.9	3.1	22.9	13.1	15.0	1.1	47.1
%BANKSHRUB	7.2	16.9	0	58.6	4.5	10.8	0	37.4
%BANKMACRO	1.7	2.6	0	8.1	2.4	4.6	0	15.7
%BANKBARE	18.5	21.2	0	67.1	29.5	30.6	0	97.1

* variables collected from 28 waterbodies (14 occupied, 14 unoccupied)

Anthoxanthum odoratum, *Lolium* sp., *Paspalum dilatatum* and *Phalaris aquatica*. The herb layer was also dominated by a variety of weed species. The vegetation at occupied sites is shown in Figures 4 – 6.

Figure 4. Survey site A1. The emergent vegetation is *Eleocharis sphacelata* with submerged and floating pondweed *Potamogeton* spp. in the foreground. Photo taken October 2005. Photo, A. Organ.

Figure 5. Survey site A3. The fringing vegetation includes *Eleocharis acuta* and *Typha,* with 'rafts' of submerged pondweed in deeper water. Photo taken October 2005. Photo, A. Organ.

Figure 6. Survey site A6. *Eleocharis sphacelata* grows at the eastern end of this large waterbody. The surrounding landscape is grazed agricultural land. Photo taken December 2005. Photo, A. Organ.

Plague minnow

The plague minnow was present at 4 of the 14 occupied waterbodies, and present at 6 of the 14 unoccupied waterbodies.

Population size and demography

Thirty-one male and 16 female *L. raniformis* were marked over the study, with an estimated male and female population size of 45 ± 10 (± S.E.) and 23 ± 8, respectively. The recapture rate for male and female *L. raniformis* in the study area was 90.3% and 81.2%, respectively. Males and females were recaptured in the waterbody where they were originally marked on up to seven and four occasions, respectively, suggesting a high degree of site fidelity exists within the population (Figure 7).

The mean SVL of male and female frogs in the study area was 66.8 ± 5.8 mm (n = 31) and 77.1 ± 9.2 mm (n = 16), respectively. The mean mass was 25.7 ± 6.0 g and 41.2 ± 14.8 g, respectively. Females were significantly larger than males (SVL: $t = -4.68$, $P < 0.001$, d.f. = 45; mass: $t = -5.10$, $P < 0.001$). Male SVL increased significantly between survey periods 1 and 3 ($F_{2,57} = 3.71$, $P = 0.03$), whereas there was no significant difference in female SVL ($F_{2,28} = 0.04$, $P = 0.96$).

The dominant male size class in survey periods 1, 2 and 3 was 70.0-74.9 mm (32.0, 34.8 and 50.0%, respectively) (Figure 8). The dominant female size classes in survey periods 1, 2 and 3 were 80.0-84.9 mm (45.4%), 70.0-74.9 mm (33.3%) and 85.0-89.9 mm (37.5%), respectively.

Breeding and recruitment

Recruitment was recorded at sites A3 and A6. Late stage tadpoles (Gosner stages 42 – 45) were first observed at site A6 on 22 December 2005, with metamorphosing frogs being observed on 28 December 2005 (n = 3), and 5 and 24 January 2006 (n = 4). One metamorph was observed at site A3 on 8 February 2006. Despite the low number of waterbodies where breeding was recorded, male frogs were recorded calling from eight waterbodies (A1, A2, A6, A9, B3, B5, C1 and C10). The plague minnow was not detected in the two waterbodies where recruitment occurred.

Movement

Three males and four females were recorded moving between sites (Table 3). One male moved 130 m from a calling aggregation at site A1 to A2, where the individual was recorded calling and resided for approximately one week, then moved back to site A1. One male moved 20 m from a non-calling site (A5) to a calling site (A6), and one moved 15 m from a calling site (C1) to a non-calling site (C2). Females moved to the nearest occupied site, between sites A5 and A6, generally within one to three weeks.

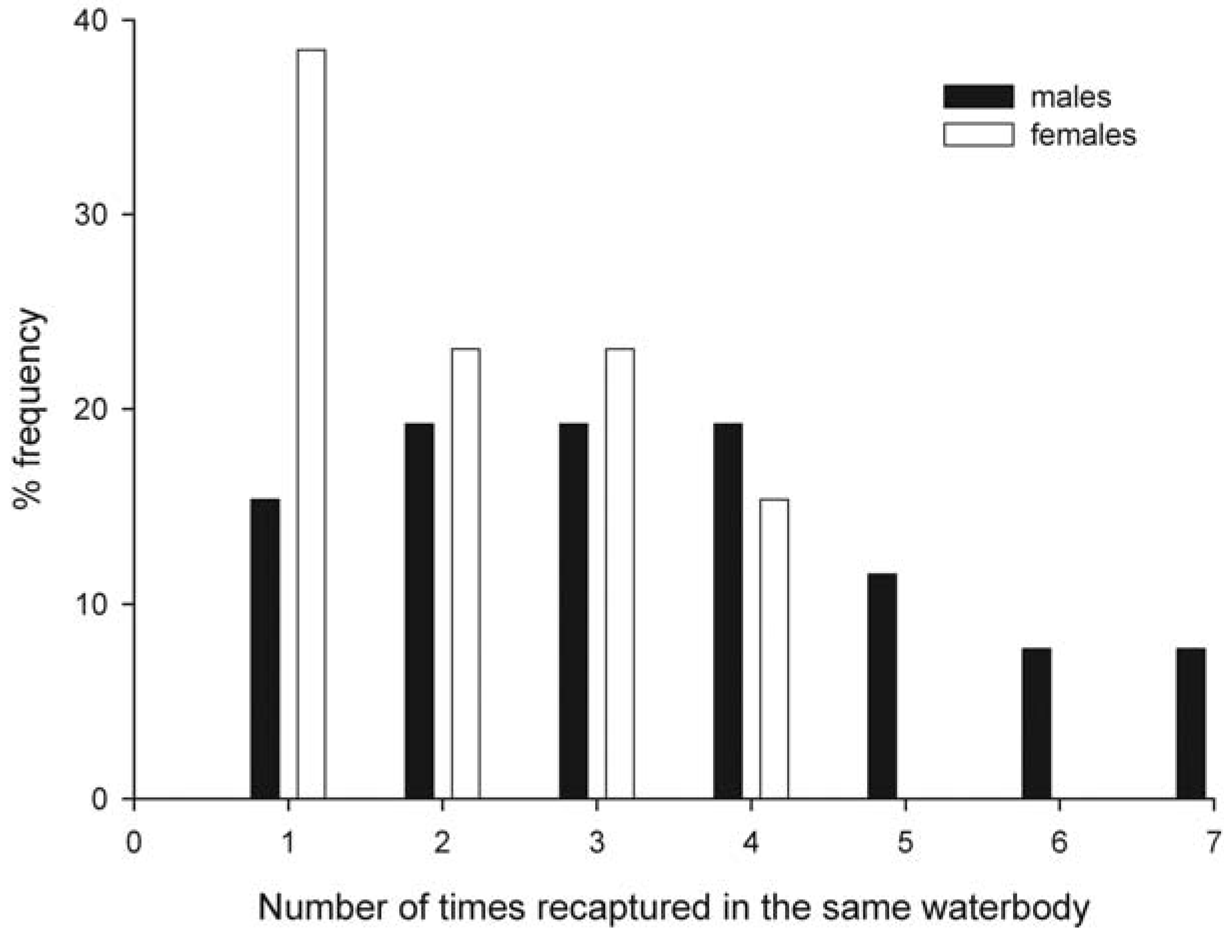

Figure 7. Frequency of male (n = 26) and female (n = 13) *L. raniformis* recaptured in the same waterbody.

Discussion

Spatial Distribution

The distribution of *L. raniformis* in the study area was clustered and a waterbody was more likely to be occupied if it was within 200 m of an occupied waterbody. This result is consistent with the spatial dynamics of many amphibian populations (Sjögren 1991; Sjögren Gulve 1994, Vos and Stumpel 1995). For example, Hamer *et al.* (2002a) demonstrated that a waterbody is more likely to be occupied by *L. aurea* if it is in close proximity (i.e. within 50 m) to an occupied waterbody. In a study of populations of *L. raniformis* inhabiting waterbodies throughout the Merri Creek catchment, Robertson *et al.* (2002) and Heard *et al.* (2004) provided evidence that waterbodies distant from occupied waterbodies had a low probability of occupancy. It is therefore likely that the spatial orientation of waterbodies is one of the most important habitat determinants influencing the presence of *L. raniformis* at a waterbody.

Habitat

Submerged vegetation influenced the occupancy of waterbodies, and occupied waterbodies had a greater proportion of *Potamogeton* spp. and *Chara* spp. This result is consistent with models produced by Robertson *et al.* (2002) and Heard *et al.* (2004) that identified submerged vegetation as a significant predictor of the distribution of *L. raniformis* within the Merri Creek catchment. *Litoria raniformis* may depend on submerged vegetation for several life-history requirements. For example, most male frogs were observed calling on rafts of submerged vegetation at the waterbody surface. Because calling activity ceased when *Potamogeton* spp. died back, it is possible that male *L. raniformis* use the presence or absence of submerged vegetation as an environmental cue to commence/end their calling activity period, although this relationship requires further investigation. Submerged vegetation is also likely to provide egg-laying sites for females, and refuge and food for tadpoles. The impact of predation on tadpoles by fish and macroinvertebrates may be reduced if a high proportion of aquatic vegetation is available (Morgan and Buttemer 1996; Hamer *et al.* 2002b). Although pH was a significant factor in the habitat model, and was higher at occupied waterbodies, high pH may provide favoured growth conditions for submerged vegetation and may not necessarily influence occupancy of waterbodies by *L. raniformis per se*.

Limitations of the Habitat Model

The low number of occupied and unoccupied sites restricted the number of independent variables that

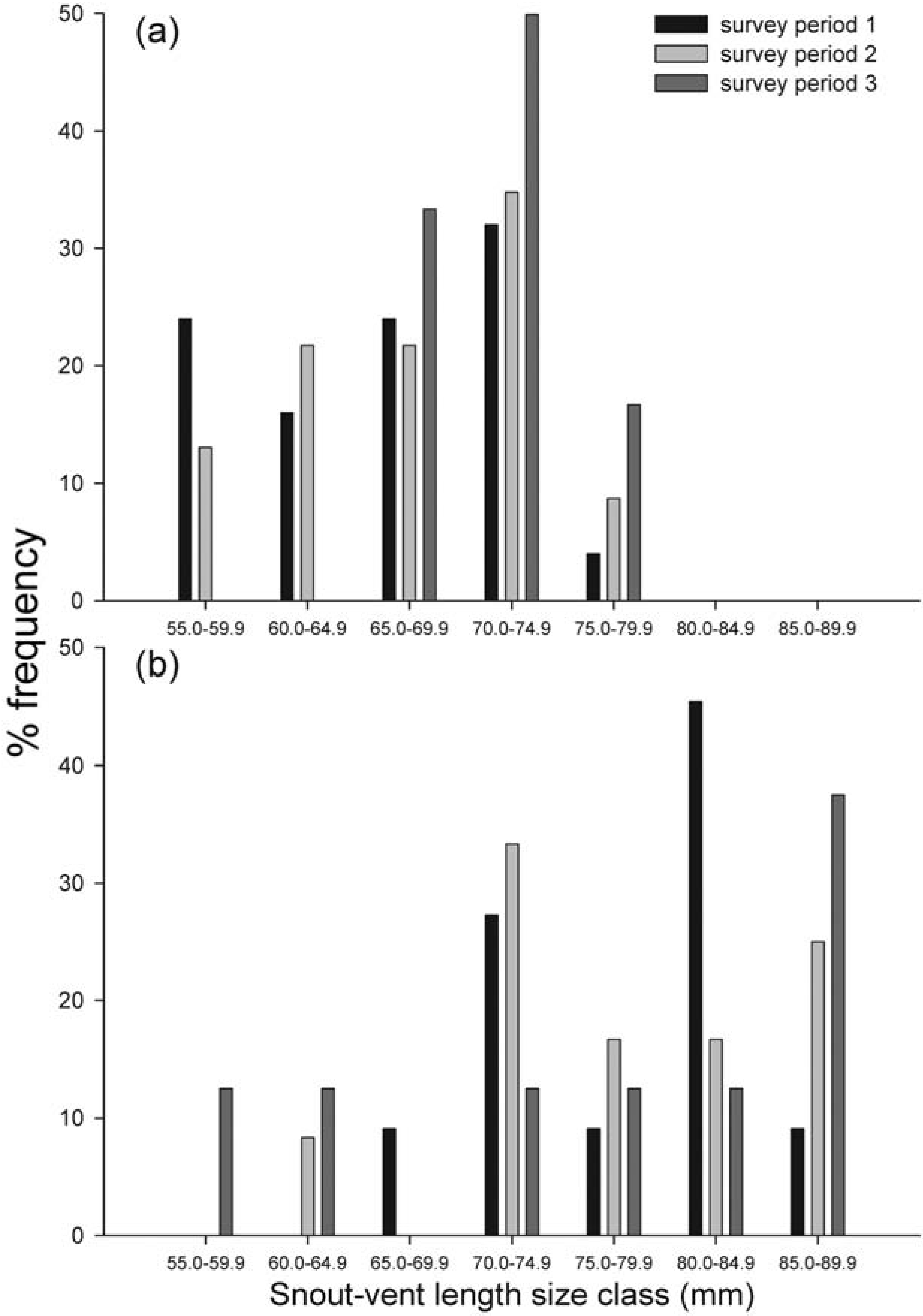

Figure 8. Frequency size (SVL) distribution of (a) male and (b) female *L. raniformis* over the three survey periods.

Table 3. Description of frog movements in the study area.

	Mean snout-vent length (mm)	Site of initial capture S1	First recapture site R1	Distance (m)	Duration (days) R1-S1	Second recapture site R2	Distance	Duration (days) R2-R1
Males	67.2	A1	A2	130	7	A1	130	9
	58.3	A5	A6	20	14	-	-	-
	70.1	C1	C2	15	37	-	-	-
Females	85.7	A5	A6	20	22	A5	20	8
	79.6	A5	A6	20	8	A5	20	23
	71.5	A6	A5	20	8	-	20	-
	81.3	A6	A5	20	23	A6	20	6

could be included in the logistic regression analysis. Therefore, the model constructed in this study should be regarded as preliminary, and frog presence/absence and habitat surveys need to be conducted at more sites in the future to build on the existing model. Furthermore, because of the close proximity of the occupied waterbodies to one another, which implies spatial dependency, and the frog movements recorded between waterbodies, the waterbodies included in the model were not independent samples. Further studies may require the inclusion of sites from a larger area.

Population Size and Demography

The population size estimate for *L. raniformis* in the study area over the 2005/06 breeding season is low compared to the number of frogs recorded at sites in previous years (Organ 2004). For example, using the maximum number of adult frogs recorded over a single night, Organ (2004) recorded 28 frogs at site C1 in December 2003, compared to a maximum of five frogs recorded in this study in January 2006. Organ (2004) also recorded 15 frogs at site A8 in December 2003, although we did not record any individuals at this site. Therefore, compared to previous seasons (e.g. 2003/04), the distribution and abundance of *L. raniformis* in the study area appeared to have decreased. This may be related to a decrease in rainfall between the two periods, because of the prevailing drought conditions in 2005/06, which may have limited breeding activity and recruitment. The male size structure probably reflects limited recruitment into the adult population, and an absence of immigration from surrounding waterbodies into the study area, resulting in larger males dominating survey period 3.

The small population size (approx. 70 individuals estimated) renders *L. raniformis* in the study area highly vulnerable to habitat loss and fragmentation, and stochastic events (e.g. drought). If waterbodies become unoccupied when they dry out, there may be few individuals available to recolonise the sites when habitat conditions improve (e.g. after heavy rain). Human impacts such as road construction and residential development will decrease the potential of unoccupied sites to be colonised due to the creation of impermeable barriers, in addition to the further loss of breeding and non-breeding habitat, which may potentially lead to extinction of the local population.

Breeding and Recruitment

Breeding and recruitment occurred in only two waterbodies and the size class distributions revealed low levels of recruitment into the adult population, suggesting there was limited reproduction in the study area during the 2005/06 breeding season. Most waterbodies appear to be reproductive sinks into which individuals (most likely juveniles) immigrate. The occupancy of many waterbodies is therefore likely to be maintained by juvenile dispersal from reproductive sources (i.e. breeding sites). For example, although no adult frogs or breeding was recorded at site A7 for the duration of the study, one juvenile was recorded there in the period following recruitment at site A6, which suggests the individual dispersed from site A6 following metamorphosis.

Waterbodies in the study area are likely to fluctuate in their "source/sink" status. It is therefore imperative that a network of waterbodies containing a range of habitats (e.g. permanent, ephemeral) and that are within close proximity (e.g. 200 m) to sources are conserved, regardless of whether recruitment has been recorded, because they may contribute to recruitment in the population over the longer term. Moreover, the close proximity of waterbodies to breeding sites is likely to increase the chance that juveniles will disperse successfully to colonise surrounding wetlands. Goldingay and Lewis (1999) provide evidence that the conservation of multiple breeding sites within a relatively small area (2.5 km radius) is very important in ensuring the viability of *L. aurea* in the Port Kembla region of New South Wales, which is highly urbanised.

Dispersal and Movement Corridors

The results of this study suggest that individuals are using the drains in the railway reserve, Gum Scrub Creek and adjoining rough grazing pasture as dispersal corridors. This is consistent with the regional distribution of *L. raniformis* in the Pakenham area which appears to be related to proximity to permanent creeks and drainage channels (Hamer and Organ 2006a). Hence, these features provide potentially important dispersal corridors for *L. raniformis*. Movement is generally more likely to occur between waterbodies that are situated in close proximity to each other, because frogs only have to move short distances. Most of the movement recorded in the study area was between two adjacent waterbodies (within 20 m). Unoccupied waterbodies may function as "stepping stones" for movement between better quality habitats, and individuals may reside at these waterbodies on a temporary basis.

There were no calling males at site A5 during the study, and given the relatively high number of females caught, this site is probably a refuge for females to shelter prior to spawning at site A6. Conversely, there were few females caught at site A6, and one of the few females caught was recorded in amplexus. It is therefore likely there is spatial partitioning of occupied habitat between male and female *L. raniformis* in the study area, which has also been proposed to explain differences in capture rates of *L. aurea* (Goldingay and Newell 2005a; Hamer and Mahony 2007). Indeed, the marked population of *L. raniformis* in the study area was one female to two males, which suggests there may be differences in habitat use, or alternatively, either a biased sex ratio in the population or differences in detectability between the sexes.

The high site fidelity of male *L. raniformis* observed in the population suggests that males prefer to remain at one waterbody over the breeding season and undertake opportunistic movements in an attempt to establish new calling aggregations. However, site fidelity is likely to be dependent on the maintenance of suitable habitat conditions at waterbodies where calling aggregations occur. If habitat conditions change (e.g. the waterbody dries out), individuals are likely to move to surrounding sites that provide a suitable refuge (e.g. permanent creeks and channels). High site fidelity has also been observed in male *L. aurea* (Hamer *et al.* in press).

Potential Threats to the Long-term Viability of the Pakenham Population

Given the expected growth in the human population in the Pakenham area over the next 30 years, the greatest threat to the population is habitat loss and fragmentation (and isolation) of remaining habitats due to residential development. Because *L. raniformis* and other bell frogs disperse widely and their populations appear to be structured according to source-sink and metapopulation dynamics (Hamer 2002; Heard *et al.* 2004; Goldingay and Newell 2005a, b), populations are likely to be vulnerable to changes in the landscape. For example, occupied waterbodies were distributed into clusters, generally within 200 m of another occupied waterbody, and most movement occurred between adjacent waterbodies. The loss of any waterbodies containing suitable habitat for *L. raniformis* will increase the distance between the remaining occupied waterbodies, and reduce the probability that individuals will successfully disperse within the population. Isolated waterbodies are at greater risk of extinction because they cannot be recolonised easily, because the distance to the nearest occupied waterbody may be beyond the dispersal distance of *L. raniformis*.

Several other threats to the viability of the population were observed during the study. For example, foxes were frequently observed at site A5 and a female frog was caught that had its left leg recently severed, possibly by an encounter with a fox. Overgrazing by cattle appeared to have reduced the water quality and extent of emergent vegetation at many waterbodies, which has been shown to influence frog species richness and occurrence in other agricultural landscapes in south-eastern Australia (Hazell *et al.* 2001; Jansen and Healey 2003). The fact that grazing occurred at most sites in the study area is likely to have attributed to grazing pressure not being a significant variable in the habitat model. Future threatening processes likely to affect the population in the study area include road mortality and indirect impacts resulting from residential development, such as increased stormwater runoff into waterbodies.

The presence of the plague minnow did not influence the occupancy of waterbodies by *L. raniformis* in the study area. This exotic fish is known to eat the eggs and tadpoles of *L. aurea*, and has been implicated in the decline of members of the bell frog complex (Morgan and Buttemer 1996; Mahony 1999; Pyke and White 2000; Hamer *et al.* 2002b). Other habitat studies have found no relationship between the presence of the fish and occupancy of waterbodies by *L. aurea*, and co-existence has been recorded at several sites (van de Mortel and Goldingay 1998; Goldingay and Lewis 1999; Hamer *et al.* 2002a). Nonetheless, breeding success is likely to be reduced at waterbodies where the fish is present, especially waterbodies with little aquatic vegetation and high fish densities. Predation risk has been shown to be greatly reduced if habitat complexity exists within the breeding pond, whereby tadpoles can seek refuge amongst rocks and submerged vegetation (Morgan and Buttemer 1996; Pyke and White 2000). Maintaining aquatic vegetation at waterbodies is likely to improve tadpole survivorship (Hamer *et al.* 2002b).

Implications for Management

Several high priority areas for the conservation of *L. raniformis* have been identified, including all waterbodies in cluster A, the Melbourne – Bairnsdale rail reserve and Gum Scrub Creek; sites B1 – B3; and sites C1, C2, C7 – C9 and C10. Because these waterbodies exist within a mosaic of terrestrial habitats that may support dispersal, the surrounding non-wetland areas also require protection. On-going monitoring of the population and habitats is required within high priority areas to provide an assessment of population stability, and further mark-recapture studies are recommended to determine movement patterns and estimate population size. Surveys for adults and tadpoles are also required at all sites to assess population status (i.e. occupancy and breeding). Management actions required to improve the long-term viability of *L. raniformis* in the study area are summarised in Table 4.

It is recommended that areas identified as high priority for the conservation of *L. raniformis* and its habitat in the study area, and any created habitat and movement corridors, are protected by an Environmental Significance Overlay via a Planning Scheme Amendment, or by designating areas for conservation. This would prevent inappropriate development from impacting the population and ensure a stringent assessment of the potential impacts of any development on *L. raniformis*.

There is potential for existing habitat to be enhanced and new wetland habitat created in close proximity (i.e. within 200 m) to occupied waterbodies. Waterbodies created within 200 m of occupied waterbodies have a high probability of being colonised by *L. raniformis*,

Table 4. Management actions recommended to improve the long-term viability of *L. raniformis* in the Pakenham area. See Hamer and Organ (2006a) for a detailed discussion of these actions.

Management Actions	Location of Implementation
Landscape-scale	
1. Protect wetland and non-wetland habitat	High priority conservation areas
2. Assess population status	All sites
3. Determine population stability	High priority conservation areas
4. Enhance quality of dispersal corridors	Melbourne – Bairnsdale rail reserve, Gum Scrub Creek
5. Create new dispersal corridors	High priority conservation areas
6. Study frog movements	High priority conservation areas
7. Initiate fox control program	All sites
Site-specific	
1. Create new wetlands	High priority conservation areas and their vicinity (i.e. within 200 m)
2. Enhance habitat quality	All sites
3. Annual monitoring	All sites
4. Mark-recapture to assess population size	High priority conservation areas
5. Restrict grazing around waterbodies	High priority conservation areas
6. Monitor water quality and fish presence	High priority conservation areas
7. Determine effectiveness of road underpasses	Wherever underpasses are constructed

providing they contain the habitat attributes identified in this study (e.g. submerged vegetation), in addition to other variables identified as being significant predictors of occupancy (e.g. permanent water level, Heard *et al.* 2004). Ideally, they should not contain predatory fish or have any potential for fish invasion. There also needs to be suitable dispersal habitat between existing and created habitat (e.g. grassland, swales).

Small ponds that act as "stepping stones" and movement corridors such as wet ditches and drains can be created to encourage frogs to disperse and colonise new waterbodies. Given the close proximity of Gum Scrub Creek (i.e. 20 m) to a known recruitment site (A6), it is likely that the creek is being used by juvenile frogs to disperse and colonise surrounding waterbodies. The long-term viability of the population of *L. raniformis* in the study area may depend on individuals dispersing along the creek to "rescue" waterbodies that have become unoccupied due to emigration.

Because it has been demonstrated that road density is negatively correlated with wetland occupancy by *L. raniformis* in the Merri Creek catchment (Heard *et al.* 2004), and by frogs elsewhere (Fahrig *et al.* 1995; Vos and Chardon 1998; Parris 2006), it is recommended that roads are not constructed in close proximity (i.e. within 200 m) to the high priority conservation areas. However, given the high urban growth occurring near these areas, this may not be feasible. In such cases, it is recommended that road underpasses be installed with frog fencing at appropriate locations under new roads proposed for construction in close proximity to wetland habitat and frog movement corridors. Elsewhere, underpasses have been shown to facilitate the movement of some amphibians under roads (Lesbarrères 2004) and are currently being constructed as part of the Pakenham Bypass to ameliorate any adverse impacts on the population of *L. raniformis* (Organ 2005b). However, they have not been proven to work with *L. raniformis* and so monitoring is required to determine their effectiveness.

If development continues in the Pakenham area without incorporating conservation areas for *L. raniformis*, including protection of high priority conservation areas, creation of additional habitat and provision of suitable wetland linkages, it is highly probable that the population will become extinct in the near future. This study has provided information on *L. raniformis* so that such a scenario can be avoided, provided future land use planning adheres to our recommendations and follows a coordinated approach involving all levels of government, land developers and wildlife managers.

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